Amendments to the Drawings:

The attached sheet of drawings includes changes to Fig. 2A-2C which includes insertion of the following text: under the title "Substrate Primer" -- (SEQ ID NO: 12)(SEQ ID NO: 13) --.

Attachment: Replacement Sheet

Annotated Sheet Showing Changes

REMARKS

Claims 1-38 are currently pending in this application. Claim 38 has been withdrawn. Claims 1-37 stand rejected.

With regard to the Restriction Requirement, Applicant hereby affirms the election of group 1, claims 1-37, with traverse. Applicant respectfully traverses this Restriction Requirement on the grounds that, as explained below, the Opdecamp et al reference does not teach the various elements of the claimed kit. Accordingly, the technical feature which does link claim groups 1 and 2 does define a contribution over the prior art.

The Office Action indicates that the present application allegedly fails to comply with the requirements of 37 C.F.R. 1.821(a)(1) and (a)(2). Filed concurrently herewith is a revised sequence listing for this application. Further, the application has presently been amended in order to include the appropriate references to the sequence listing.

Claims 19, 21, and 26 stand rejected under 35 U.S.C. 112, second paragraph for allegedly failing to particularly point out and distinctly claim the invention. Claims 19, 21, and 26 have been amended in order in accordance with the suggestions in the Office Action. As such, this rejection has been obviated.. Prior to discussing the rejection under 35 U.S.C. 102 and 103(a) in detail, Applicant believes it would be useful to set out the meaning of a number of the terms used in the specification of the present application. In particular:

- "Cytosine" is a pyrimidine base
- "Cytidine" is a cytosine base linked to a pentose sugar where the pentose sugar is ribose (D-ribose) to form a ribonucleoside (for RNA).
- "Deoxycytidine" is a cytosine base linked to a pentose sugar where the pentose sugar is a deoxyribose (2-deoxy-D-ribose) to form a deoxyribonucleoside (for DNA)
- A nucleoside is a purine or pyrimidine base attached to a ribose sugar.
- A nucleotide is a phosphate ester of a nucleoside. So in DNA the moiety containing the cytosine base is referred to as a deoxynucleoside.
- The bond that holds nucleosides together in DNA or RNA is a phosphodiester bridge where the 3'-hydroxyl of the sugar moiety of one ribonucleoside is joined to the 5'-hydroxyl of the adjacent sugar by a phosphodiester bond
- Restriction enzymes recognize a specific DNA sequence
- Restriction enzymes do not modify purine or pyrimidines, rather they cleave the phosphodiester bond, between the sugar moieties, that links nucleosides together. There is no modification of the purine or pyrimidine bases.
- With methylation sensitive restriction enzymes, cleavage of the phosphodiester bond is dependent on the methylation status of cytosine, but there is still no modification of the pyrimidine base (cytosine) by the restriction enzyme.
- Restriction enzymes only cleave double stranded DNA, they do not cleave single stranded DNA

- Cleavage of DNA by a restriction enzyme may result in "sticky ends" which provide a small area of single stranded DNA. This single stranded DNA is not cleaved by restriction enzymes.
- During PCR amplification, no alkylated cytosines are introduced into PCR amplicons. Standard PCR reactions contain only deoxycytidine and not 5-methyl-deoxycytidine. Even if 5-methyl-deoxycytidine were included in the PCR mix with deoxycytidine, the DNA polymerase would not copy the methylation pattern of the genomic or mitochondrial DNA target as DNA polymerases have no ability to discriminate between deoxycytidine and 5-methyl-deoxycytidine.

Claims 1-3, 14, 19, 22-25, 27-33, 35, and 37 stand rejected under 35 U.S.C. 102(b) for allegedly being anticipated by Opdecamp et al. Applicant respectfully traverses this rejection.

As described in the Office Action, Opdecamp et al teaches identification of methylated DNA by using methylation-sensitive restriction enzymes. The claims of the instant application have been amended to make it clear that in the claimed method the enzyme is acting on single stranded DNA. It is well understood that restriction enzymes only recognize and cleave double stranded DNA. Accordingly, none of the enzymes referred to in Opdecamp differentially modify cytosine and alkylated cytosine in the single stranded DNA.
Further, there is another major difference between the invention claimed in the current application and the disclosure of Opdecamp et al. As described above, cytosine is the pyrimidine base. A restriction enzyme does not modify purines or pyrimidines. Restriction enzymes cleave the phosphodiester bond between sugar

moieties that links nucleosides together. There is no modification of the purine or pyrimidine bases. Accordingly, Opdecamp et al does not disclose the use of an enzyme which differentially modifies alkylated cytosine or cytosine. Therefore, Opdecamp et al does not anticipate the claims of the current application. Thus, Applicant respectfully requests withdrawal of this rejection.

Claims 1 and 4 -3 stand rejected under 35 U.S.C. 103(a) over Opdecamp et al as evidenced by Griffith et al in view of Kuhn et al. Applicant respectfully traverses this rejection.

As discussed above, Opdecamp et al provides no disclosure at all of modifying cytosine. In particular, there is no disclosure of modifying cytosine present in single stranded DNAs as is required by the claims as amended. Kuhn et al does not overcome this deficiency.

As is noted in the Office Action, Kuhn et al provides information regarding separating two strands of double stranded DNA with different means including strand displacing probes. Kuhn et al does not, however, provide any teaching or information regarding differentially modifying alkylated cytosine and cytosine present in single stranded DNA. Accordingly, even if the skilled artisan was to combine the references, as suggested in the Office Action, they would not arrive at the current invention. As such, the combined references do not render the claimed invention obvious. Thus, Applicant respectfully requests withdrawal of this rejection.

Claims 1, 14 – 17, 22, 23 and 26 stand rejected under 35 U.S.C. 103(a) over Opdecamp et al as evidence by Griffith et al in view of Gitan et al. Applicant respectfully traverses this rejection.

As discussed above, Opdecamp et al does not disclose any modification of cytosine or alkylated cytosine in any form. In particular, there is no disclosure of modifying alkylated cytosine or cytosine in single stranded DNA. This deficiency is not overcome by Gitan et al.

Gitan et al discloses a method known as methylation specific oligonucleotide microarray. This method uses bisulphate-modified DNA as a template for PCR amplification. Bisulphite-modification results in conversion of unmethylated cytosine but not methylated cytosine into thymine within CpG islands of interest in the PCR product (see abstract). There is no disclosure in Gitan et al of the use of an enzyme which differentially modifies methylated cytosine and unmethylated cytosine in single stranded DNA. Accordingly, the present invention is not arrived at by a combination of these references.

Further, it would not be possible to combine Opdecamp's method of methylation detection with the method of Gitan et al. Opdecamp's method of methylation detection uses the methylation sensitive restriction enzyme HpaII. Once the relevant region of DNA has been cleaved by HpaII, it would be impossible to amplify the target region with PCR as required by Gitan. Accordingly, it is not possible to combine these two disclosures in the manner suggested by the examiner. Further, it is not possible to amplify the DNA according to the method of Gitan prior to digestion. If the DNA was amplified

prior to digestion, there would be no methylated cytosines and thus no differential target for the enzyme HpaII. It is therefore not possible to combine the method of Opdecamp et al with the PCR method of Gitan et al to perform methylation detections. As such, Applicant respectfully requests withdrawal of this rejection.

Claims 1 and 34 stand rejected under 35 U.S.C. 103(a) over Opdecamp et al as evidenced by Griffith et al in view of Paulson et al.

As discussed above Opdecamp et al does not disclose differential modification of methylated cytosine and cytosine in single stranded DNA. This deficiency is not overcome by the disclosure of Paulson et al.

Paulson et al uses bisulphite modified DNA which is then amplified using PCR to detect sites of methylation in the viral genome. Accordingly, the methodology of Paulson et al is similar to the methodology of Gitan et al discussed above. There is no disclosure in Paulson et al of use of an enzyme which differentially modifies cytosine present in single stranded DNA. In addition, for the reasons discussed above in relation to Gitan et al it is not possible to combine the methods of Opdecamp et al and Paulson et al. As such, Applicant respectfully requests withdrawal of this rejection.

Claims 1, 18, 20 – 21 and 35 – 36 stand rejected under 35 U.S.C. 103(a) over Chaudhuri et al in view of Bransteitter et al.

Neither Chaudhuri or Bransteitter disclose methods of detecting the presence or level of alkylated cytosine in a sample of DNA.

In Chaudhuri et al, the double stranded DNA substrate is generated by amplifying by PCR, a chemically produced 60-base oligonucleotide that has been inserted into a plasmid. The PCR product is then rendered single stranded by heating to 100 degrees and cooling on ice. It is after this PCR amplification step that Chaudhuri adds AID to the reaction to modify cytosines to uracil. The method of Chaudhuri et al of amplification by PCR prior to treatment with AID does not teach any ability to differentially modify alkylated cytosine and cytosines as there no alkylated cytosines in the PCR product. Accordingly, this method does not provide any way of detecting the presence or level of alkylated cytosine in a sample.

This deficiency is not remedied by Bransteitter. Bransteitter does not disclose measuring the presence or level of alkylated cytosine in a sample. Accordingly, even if it was assumed that the skilled addressee would combine these two references they would not arrive at the claimed invention. As such, Applicant respectfully requests withdrawal of this rejection.

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket #102782.60031US).

Respectfully submitted,

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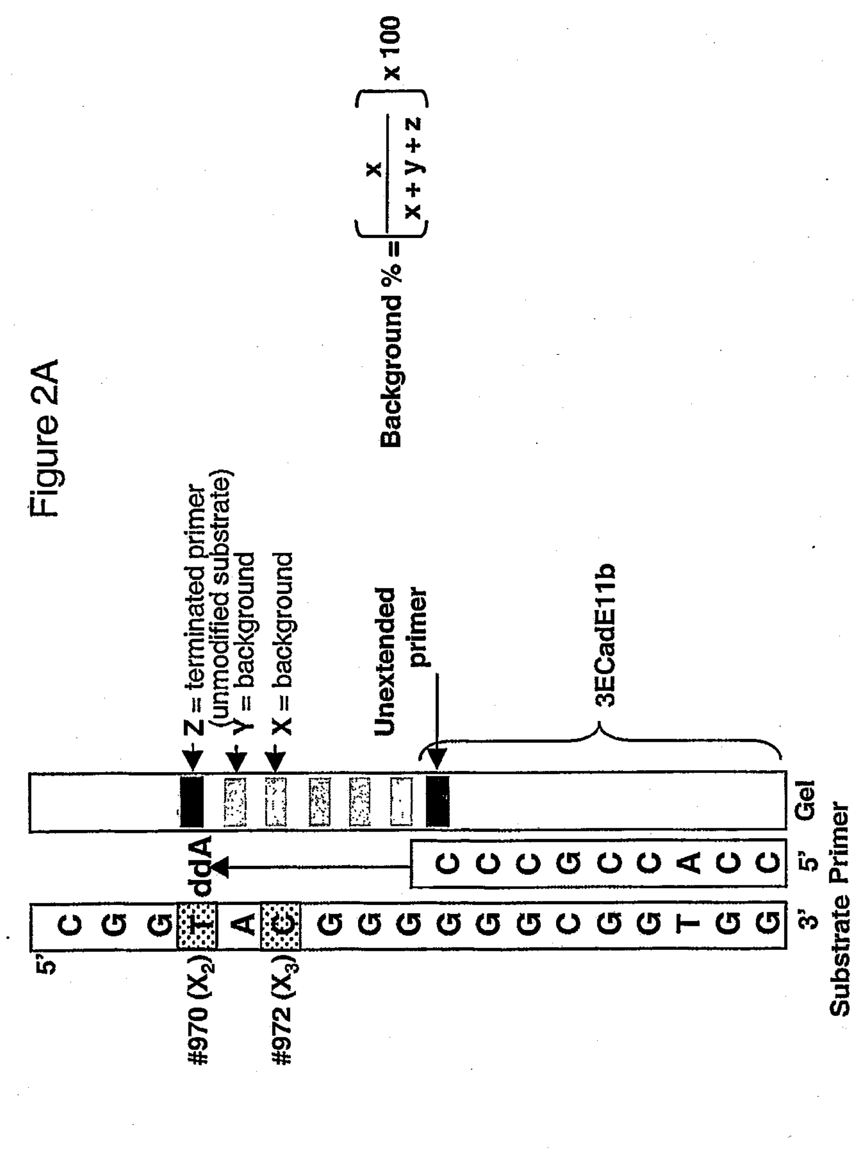
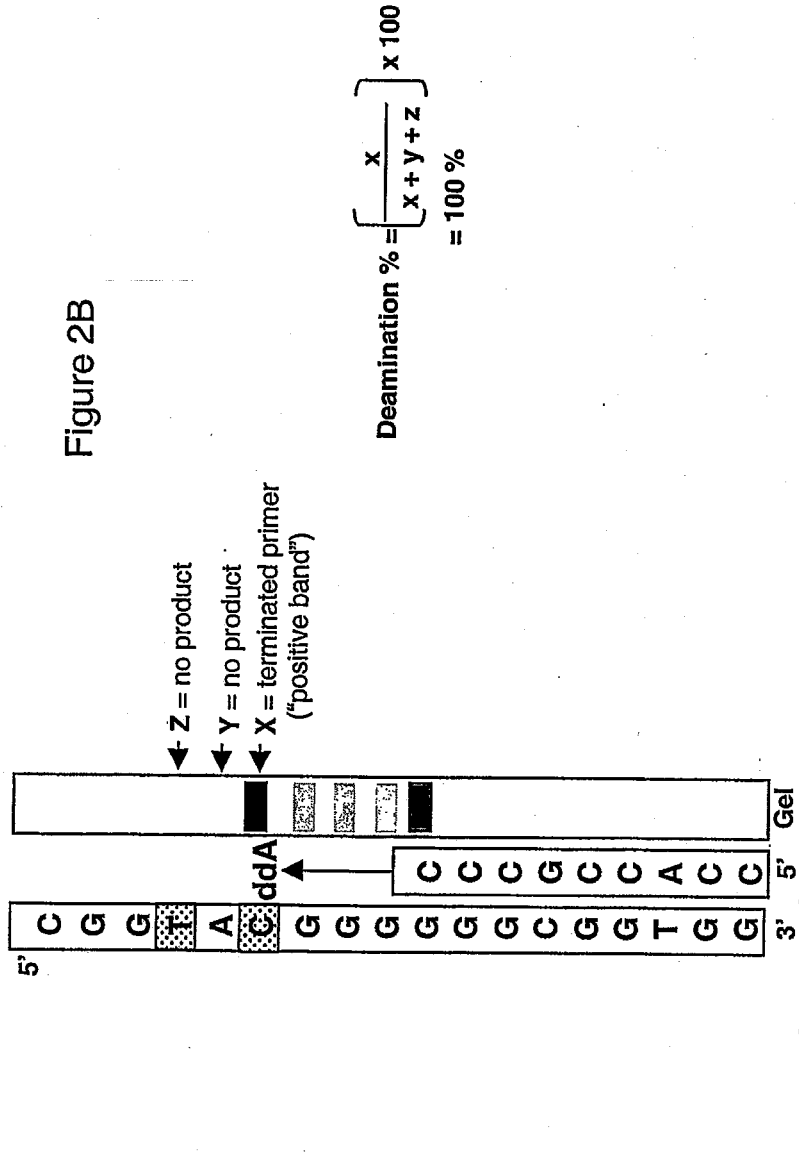
Figure 2A



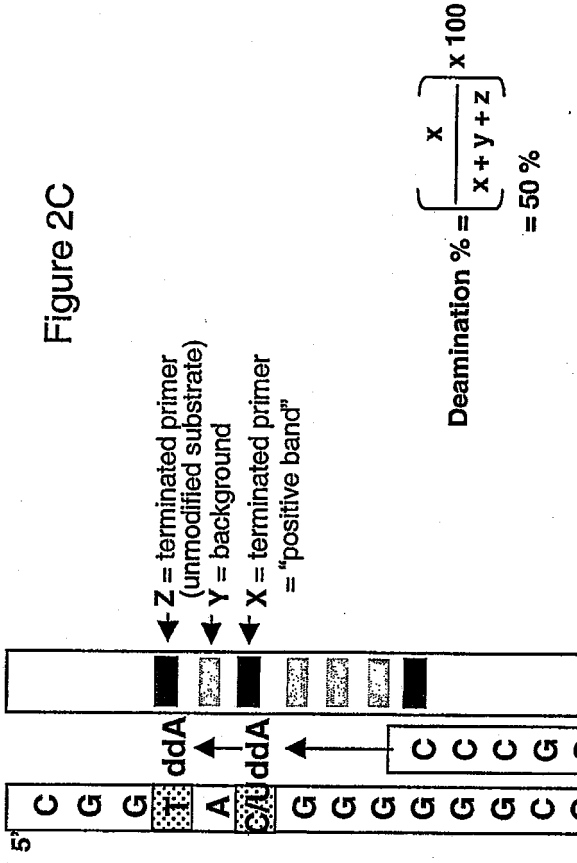
--(SEQ ID NO:12)(SEQ ID NO:13)--

Figure 2B



-- (SEQ ID NO: 12)(SEQ ID NO: 13) --

Figure 2C



-(SEQ ID NO:12)(SEQ ID NO:13)--